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Modulation of apoptosis by ischemic preconditioning: an emerging role for miR-21

Tarek M. El-Achkar[1]

Ischemic preconditioning (IPC) is a powerful phenomenon whereby an episode of ischemic injury protects the kidney from subsequent injury. Xu *et al.* provide new insights into the protective effects of delayed IPC and its inhibition of apoptosis by implicating a modulatory role for the microRNA miR-21. This study adds another layer to our understanding of IPC, but also hints at the complexity of the system triggered by this process.

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Acute kidney injury caused by ischemia is a major cause of mortality and morbidity in hospitalized patients. Interventions to reduce the incidence or severity of AKI could potentially have a significant impact on patient care. Guided by old observations, it has been appreciated over the past few decades that an episode of ischemic kidney injury may confer protection from subsequent injury, a phenomenon known as ischemic preconditioning (IPC).[1] While the protective effects of IPC may occur as early as 15 min after the initial ischemic event,[2] they can also be delayed, persisting as late as 12 weeks in some experimental conditions.[3] For various reasons, it is difficult to estimate the occurrence and importance of IPC clinically. In theory, IPC could be a relatively common phenomenon. For example, patients who develop transient AKI during prolonged hospitalization could be benefiting from IPC and become immune to subsequent insults. IPC could also be an important player in transplantation, in which ischemia–reperfusion is a starting point for all transplanted kidneys. In addition, because of its protective and delayed nature (that is, minimizing subsequent deviation from normal), the occurrence of IPC will always be underestimated. Nevertheless, and despite its potential clinical relevance, IPC is still not well understood. Furthermore, unraveling the cellular and molecular mechanisms underlying the protective effects of IPC is extremely important, because it could allow the development of therapeutic approaches that modulate these same key pathways independently of IPC.

Multiple mediators and pathways may be important in renal IPC, summarized in Figure 1. IPC also promotes a state of peripheral immunosuppression and recruitment of anti-inflammatory lymphocytes (regulatory T cells) to the kidney through unclear mechanisms.[4,5] Early protection and delayed protection conferred by IPC share common signaling pathways, such as activation of pertussis toxin-sensitive G protein and protein kinase C.[2,6] However, there may also be time-dependent differential activations of separate pathways, which could explain the various waves of protection conferred by IPC. For example, free radical generation and Akt activation play a role in early but not in delayed IPC.[2] Inducible nitric oxide and the increased expression of heat-shock proteins could be important mediators of late preconditioning.[2,3,7] Inhibition of JNK/p38 activation could also be an important determinant of a delayed IPC.[7] This is relevant because JNK activation could be part of a pro-apoptotic signal in AKI.[8] Although these studies have been essential in constructing the chain of molecular events triggered by IPC, it is not clear whether these signaling events establish a redundancy in the system or whether a cross-talk exists between these various pathways.

Xu *et al.*[9] (this issue) add another dimension to our understanding of the molecular mechanisms implicated in delayed IPC. They studied the role of microRNAs in IPC. MicroRNAs (miRNAs) are short non-coding RNA sequences that regulate gene expression by base-pairing with specific mRNAs predominantly at their 3′-untranslated region, thereby regulating their stability and translation.[10] Several enzyme complexes are involved in this process; for instance, Dicer complex cleaves a pre-miRNA precursor. Argonaute and other proteins bind the cleaved miRNA to form the RNA-induced silencing complex (RISC). Depending on the degree of base pair matching with the corresponding mRNA, RISC can either lead to its degradation or repress its translation. The role of miRNAs in kidney diseases in general and specifically AKI is not fully clear. Wei *et al.* from Zheng Dong's laboratory showed that targeted deletion of Dicer from proximal tubules was associated with protection from AKI, supporting a role for miRNA in modulating AKI.[11] Several miRNAs appear to be differentially expressed by ischemia.[11,12] Among these, miR-21 appears to have antiapoptotic properties, in part by targeting the expression of programmed cell death protein 4 (PDCD4). In fact, using isolated primary renal cells *in vitro*, Godwin *et al.* recently showed that miR-21 upregulation by ischemia was associated with decreased PDCD4 expression and increased

[1]*Division of Nephrology, Indiana University and the Richard L. Roudebush Veterans Affairs Medical Center, Indianapolis, Indiana, USA*
Correspondence: *Tarek M. El-Achkar, Indiana University, Division of Nephrology, 950 W. Walnut Street, R2-202, Indianapolis, Indiana 46202, USA. E-mail: telachka@iupui.edu*

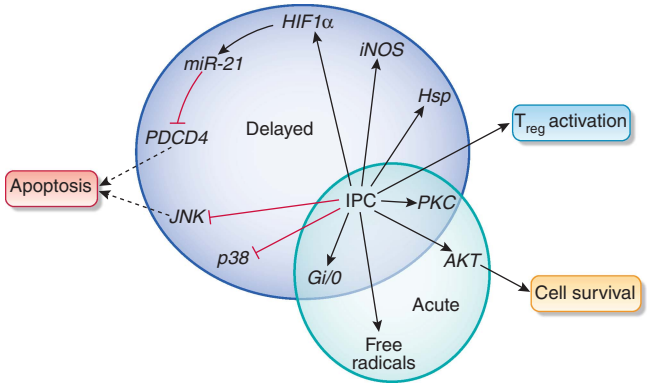

Figure 1 | Signaling pathways triggered by ischemic preconditioning. A summary of the potential pathways triggered by ischemic preconditioning (IPC) is presented. Few signaling cascades are common to early and delayed IPC (intersection between the two Venn diagrams), whereas other pathways are more differentially associated. The novel findings by Xu *et al.* suggest that IPC stabilizes hypoxia-inducible factor-1α (HIF1α), which in turn increases the transcription of miR-21. The latter inhibits the translation of programmed cell death protein 4 (PDCD4), thereby having an overall role of inhibiting apoptosis. Other abbreviations: iNOS, inducible nitric oxide synthase; PKC, protein kinase C; T_{reg}, regulatory T cell.

bcl-2.[12] Knock-down of miR-21 *in vitro* was associated with decreased survival of renal cells, and its overexpression prevented their basal rate of death. However, when these cells were subjected to ischemia, miR-21 overexpression was not sufficient to prevent cell death.[12]

In this current work, Xu *et al.* show that delayed IPC reduces the susceptibility of the kidney to AKI and tubular apoptosis.[9] They used a common model of IPC[7] in which ischemia–reperfusion was performed 4 days after IPC. The authors show that miR-21 expression is increased shortly after IPC and remains elevated throughout the reperfusion period. They also report that other miRNAs, such as miR-320, miR-214, and let-7e, were not significantly increased by IPC. Using locked nucleic acid oligonucleotide inhibitor, they show that miR-21 knock-down *in vivo* after IPC aggravated AKI and tubular apoptosis compared with non-specific oligonucleotide, suggesting a role for miR-21 in IPC. Interestingly, miR-21 did not affect the severity of AKI in the absence of IPC. The protection conveyed by miR-21 could be explained partially by its inhibitory effect on PDCD4. Indeed, PDCD4 expression increased when miR-21 was knocked down after IPC. Finally, the authors also show[9] that increased levels of hypoxia-inducible factor-1α (HIF1α) that occur and persist after IPC could be the link to the upregulation of miR-21.

This study is important for various reasons. First, it provides additional evidence for the importance of miRNAs in regulating the response of the kidney to injury *in vivo*. In addition, this work underscores the importance of the antiapoptotic effects of delayed IPC, which could also complement the findings by the Bonventre group on inhibition of JNK/p38 by IPC,[7] although Xu *et al.* did not find a difference in the activation of JNK by anti-mir-21 treatment.[9] The work by Xu *et al.* also identifies a potential pathway that links IPC to inhibition of apoptosis via a HIF1α–miR-21–PDCD4 axis (Figure 1). The role of HIF1α in regulating the transcription of miR-21 is a novel finding, and supports a general role for HIF1α as a transcription factor for various miRNAs.[10] Finally, this study generates excitement from a technical aspect, whereby locked nucleic acid oligonucleotides are successfully used *in vivo* to knock down mir-21. The use of this technology to manipulate the levels of miRNA *in vivo* could become quite valuable in the study of miRNAs.

The study also has limitations. The degree of injury after ischemia–reperfusion seen by histology and functionally through measurement of serum creatinine appears to be mild compared with that in similar work done by others, such as Joo *et al.*[2] The authors address this by citing variability due to technical and operator-dependent factors, which could partially explain the discrepancy. However, because the severity of kidney injury could affect differential pathways,[3] it will be important to verify whether the role of miR-21 is limited to mild injury, or whether its modulatory role could also be appreciated in more severe insults. To their credit, the authors demonstrated the reproducibility of the findings in their model with a time course extending up to 5 days after ischemia–reperfusion.

If kidney injury and apoptosis are aggravated by miR-21 knock-down after IPC, does this mean that overexpression of mir-21 after IPC is beneficial? The study by Xu *et al.*[9] was not designed to address this question. In fact, the findings by Godwin *et al.* that renal cells overexpressing miR-21 were not protected after ischemia could argue against this.[12] In addition, Xu *et al.* also show that inhibition of miR-21 is detrimental only after IPC,[9] suggesting that other factors induced by IPC are essential for the actions of miR-21. Therefore, future investigations are needed to clarify whether the role of miR-21 in IPC is permissive, acting as an on/off switch, or whether the level of miR-21 achieved after IPC correlates with its antiapoptotic effects. In addition, studies will also need to identify what factors induced by IPC are essential for the activity of miR-21, and what is the relationship of miR-21 to the pathways already established for IPC.

In conclusion, this study by Xu *et al.*[9] uncovers a novel modulatory role for miR-21 in IPC, and a potential link between IPC and inhibition of apoptosis, involving a HIF–miR-21–PDCD4 axis. The study also underscores the complexity of the system triggered by IPC, and hints at the multiple challenges facing the transition

from understanding molecular mechanisms of IPC to therapeutic applications. A therapeutic approach to AKI will probably be multifaceted, targeting multiple pathways at various times. IPC is a very powerful phenomenon, specifically because it can trigger multiple layers of (protective) signaling pathways. Therefore, understanding the interactions between various signaling pathways and the modulatory role of miRNAs in IPC will no doubt be essential.

DISCLOSURE
The author declared no competing interests.

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Strategies to reverse endothelial dysfunction in diabetic nephropathy

Shawn S. Badal[1,2] and Farhad R. Danesh[1,2,3]

Endothelial dysfunction underlies the basic pathophysiology of microvascular complications of diabetes. Endothelial dysfunction is associated with impaired nitric oxide (NO) availability. Since NO production is tightly regulated by endothelial nitric oxide synthase (eNOS), several therapeutic strategies have been investigated and proposed to improve eNOS bioavailability in the vasculature. The findings of Cheng *et al.* suggest that increased availability of eNOS may be an effective strategy in restoring endothelial function in patients with diabetic nephropathy.

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The critical role of endothelial dysfunction in micro- and macrovascular complications of diabetes has generated considerable interest in identifying strategies to improve endothelial function in the diabetic milieu. An interesting study by Cheng *et al.*[1] confirms the importance of endothelial nitric oxide synthase (eNOS) activity in endothelial dysfunction, and reports that improving eNOS activity ameliorates progression of diabetic nephropathy. This work provides evidence for a key role of endothelial dysfunction in the progression of diabetic nephropathy, and supports a rationale for pharmacological targeting of the eNOS pathway as a novel strategy in the treatment of diabetic kidney disease.

The endothelium is not only a monolayer of endothelial cells that lines the entire vascular system, but also exerts significant autocrine, paracrine, and endocrine actions modulating vasodilation, smooth muscle cell growth and migration, and inflammatory responses. Many of these effects are mediated by nitric oxide (NO). NO opposes the effects of endothelium-derived vasoconstrictors such as angiotensin II and endothelin, protects against endothelial-cell damage induced by cytokines such as tumor necrosis factor, and provides antithrombogenic effects by blocking the release of von Willebrand factor. Indeed, a defect in the production or activity of NO promotes key features of endothelial dysfunction, such as vasoconstriction, platelet

[1]*Division of Nephrology, Baylor College of Medicine, Houston, Texas, USA;* [2]*Interdepartmental Graduate Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, Texas, USA and* [3]*Department of Pharmacology, Baylor College of Medicine, Houston, Texas, USA*
Correspondence: *Farhad R. Danesh, Department of Medicine, Division of Nephrology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA. E-mail: danesh@bcm.edu*